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Title:	Opportunities for the capture and utilization of CO2 by biological platforms
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Opportunities for the capture and utilization of CO_2 by biological platforms

Raul Gonzalez

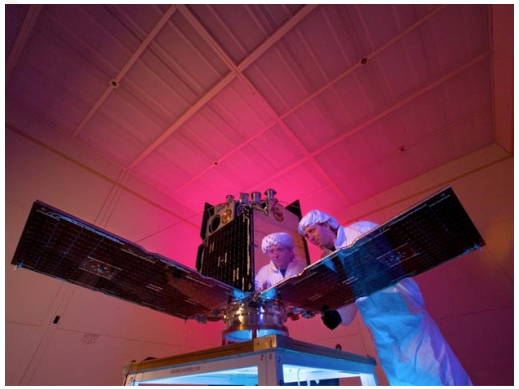
Scientist 2

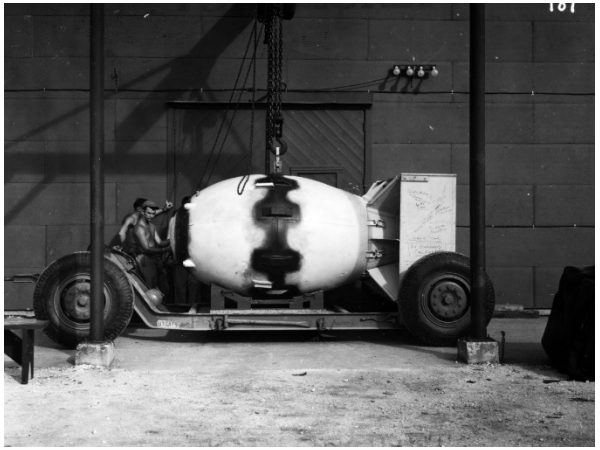
Scientific Background

- "Licenciatura" Chemist-Pharmacobiologist (UAG)
  - Antioxidant response in banana trees after mechanical wounding
- "Scholar" (Jose Cuervo Agriculture Division)
  - Biochemical signatures leading to high biomass in *Agave tequilana.*
- PhD in Plant Biology (Arizona State University)
  - Developing cyanobacteria as platforms for biofuel production (metabolic engineering and heat shock tolerance)
- 1st Postdoc (Michigan State University)
  - Engineering of the CO_2-concentration mechanism of cyanobacteria (synthetic biology and physiology)
- 2nd Postdoc (LANL)
  - Engineering of the CO_2-concentration mechanism of microalgae; development of algae transgenics for biofuel production

Los Alamos National Laboratory

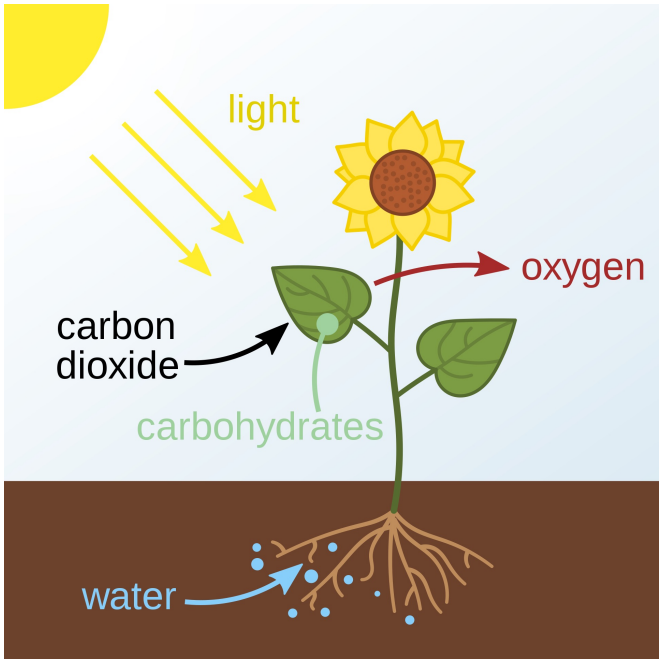
Mission:
Solve national security challenges through scientific excellence.





Photosynthesis

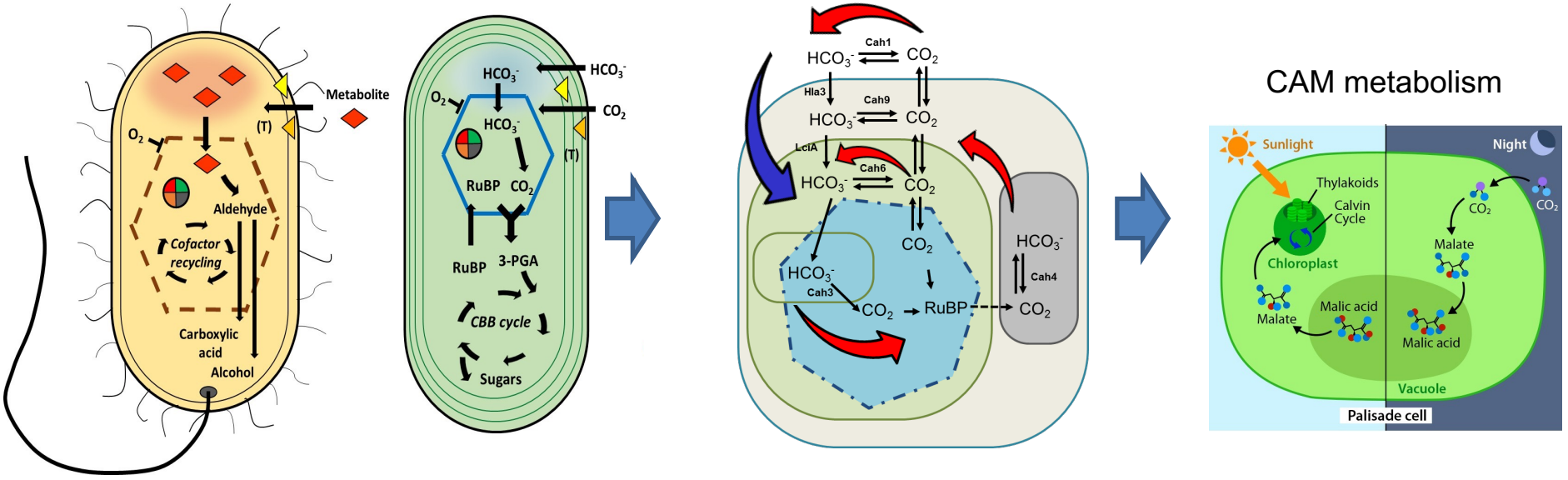
Carbon dioxide+ water + sunlight⇒
oxygen + sugars



SynBio as the bridge between interdisciplinary research

Prokaryotes

Eukaryotes



Bacteria

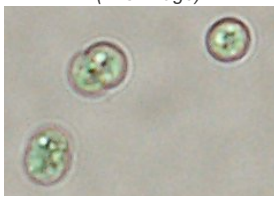
Cyanobacteria

Microalgae

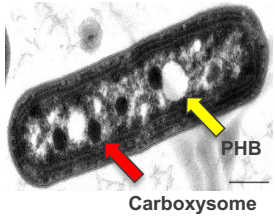
Photosynthetic production of renewable chemicals

- Cyanobacteria are fast growers, genetically tractable and possess highly efficient CO_2-concentrating mechanisms (bicarbonate pumps and carboxysomes).

Synechocystis sp. PCC 6803
(DIC image)

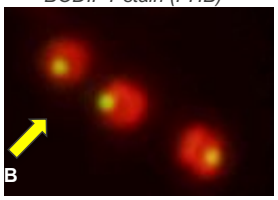


Synechococcus elongatus
(TEM micrograph)

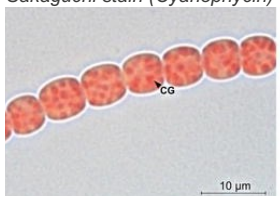


- Cyanobacterial metabolism is a vast resource for bioactive molecules and renewable polymers, many yet to be characterized.

BODIPY stain (PHB)



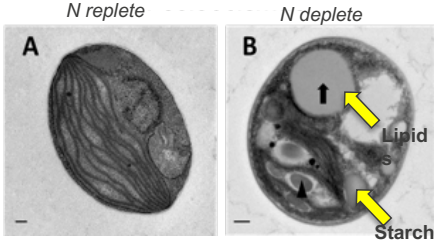
Sakaguchi stain (Cyanophycin)

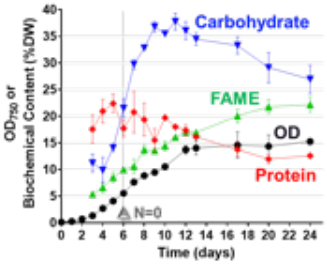


- Polyphosphates
- Microcystins
- Phyto-hormones
- Siderophores
- Pigments
- ??

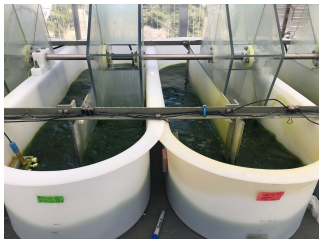
- Microalgae utilize their fixed CO_2 towards the accumulation of C-rich storage molecules (during nutrient stress).

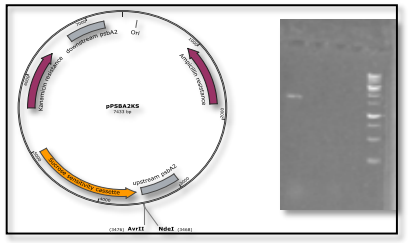
Picochlorum soloecismus





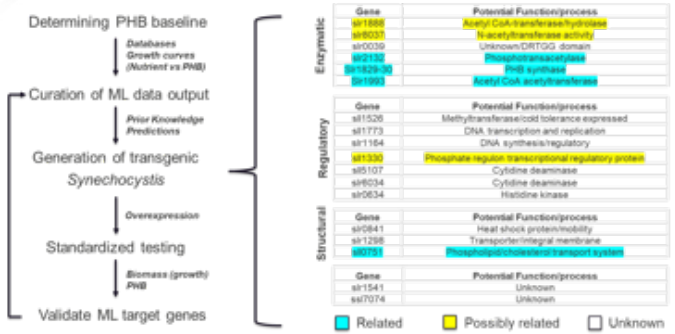
- Microalgae can be grown outdoors efficiently, and can be genetically-engineered.





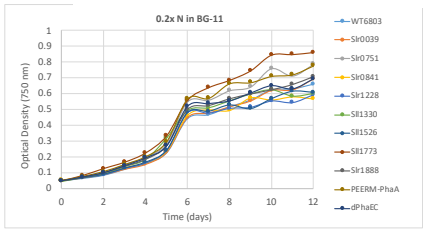
BioManiac: Biomanufacturing with Intelligent Adaptive control

- ML-based method for the discovery of genes that are non-intuitively related to PHB (bioplastic) synthesis in cyanobacteria

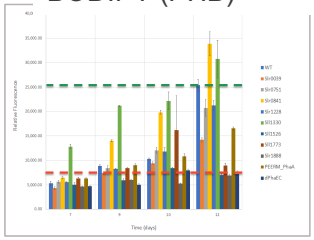


✓ ***Various transgenics show improved cell growth or altered PHB***

CELL GROWTH

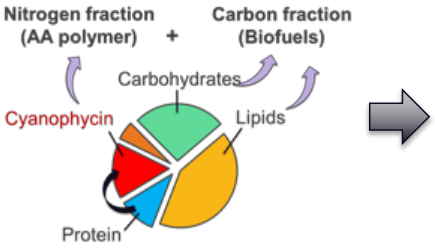


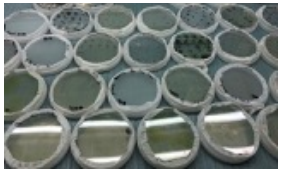
BODIPY (PHB)



Engineering green factories for the production of renewable chemicals

- Add value to microalgal biomass, through the co-production of C-rich storage molecules and the N-based cyanobacterial polymer, cyanophycin (replacement for absorbents, paints, adhesives, detergents)

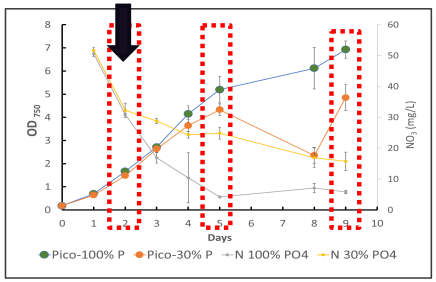


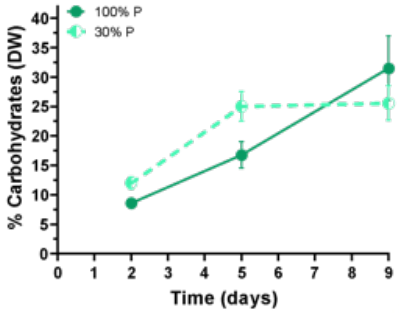


✓ Transgenic algae expressing cyanobacterial gene (CphA)

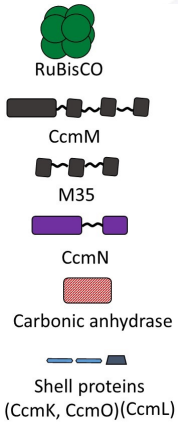
✓ ***Phosphate limitation induces C storage***

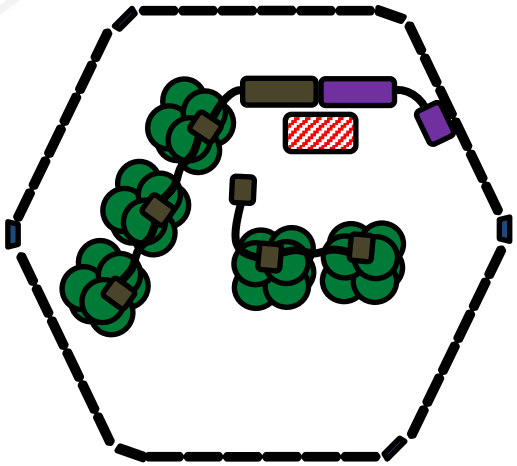
New storage trigger: P≈0 in media



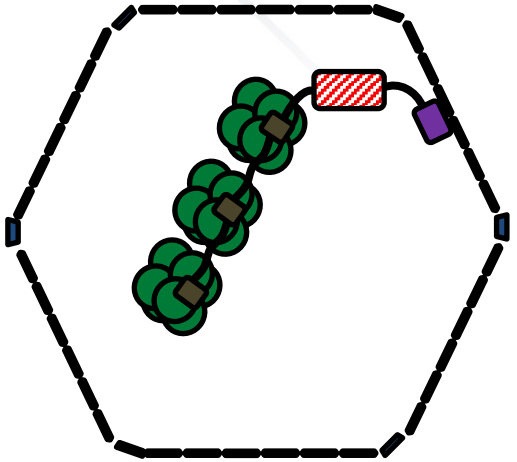


Comparison of wildtype and mutant carboxysomes





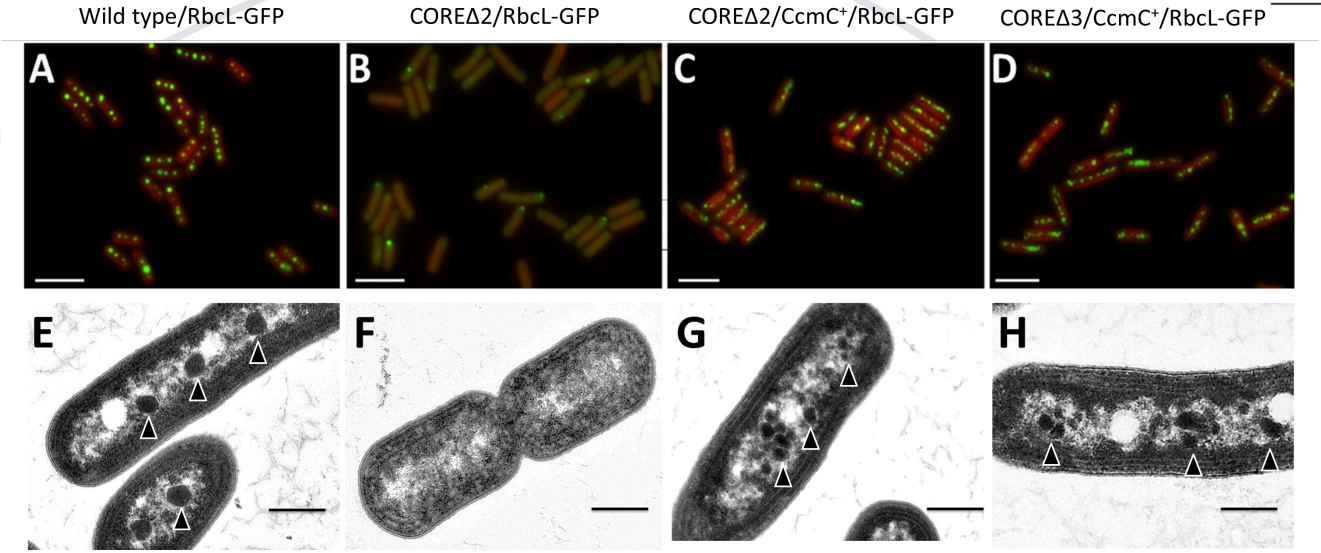
Wildtype carboxysome

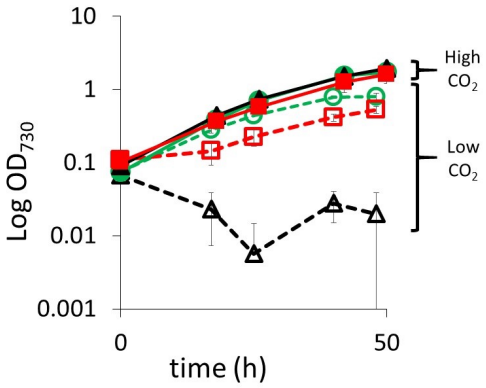


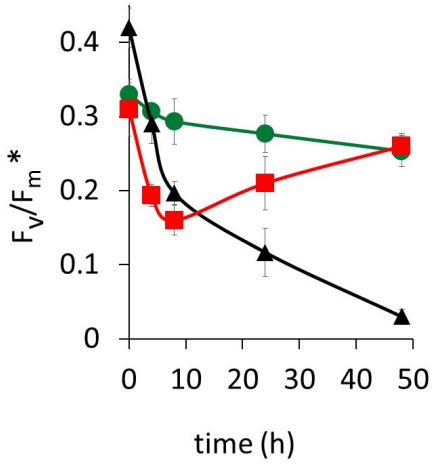
Streamlined carboxysome

- Flexibility of subunits under environmental conditions
- Several subunits required for transfer to other organisms
- Complex assembly and regulation in other organisms

- Fixed stoichiometry of the composing domains
- Increased portability (fewer number of proteins required)
- Simplified assembly and regulation (one core assembly protein) in other organisms
- Potential for improvement





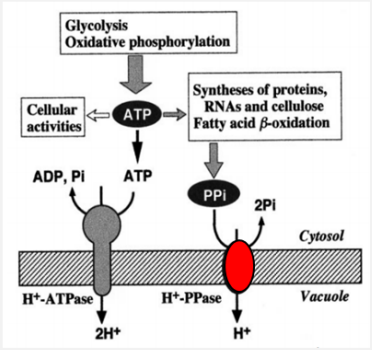


Wild type/RbcL-GFP
COREΔ2/RbcL-GFP
COREΔ3/CcmC/RbcL-GFP

* Upon change from 5% CO_2 to a

Increasing Carbon Storage in Transgenic Algae

Overexpression of a H^+-pumping pyrophosphatase (AVP1)

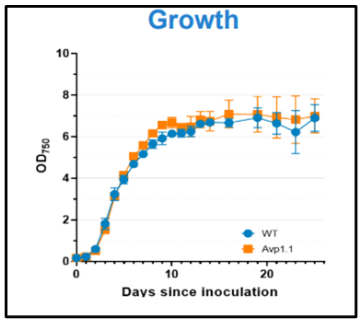


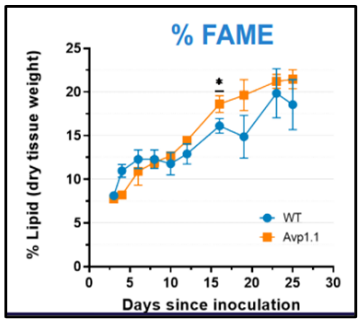
Maeshima, M. (2000).

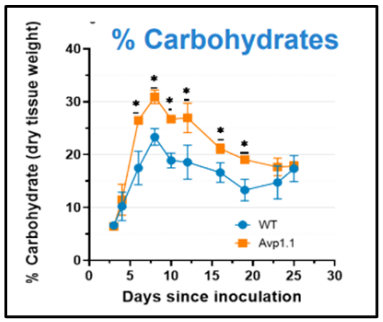
- **Avp1 in plants:**
  - PP_i homeostasis (metabolic regulation)
  - Creates H^+ gradient for ATP synthesis
  - Phloem loading/sucrose transport
- **Avp1 overexpression:**
  - Increases biomass, salt tolerance, and drought tolerance in crops like cotton, tomato, rice, and wheat.

➢ ***Avp1 overexpression effect unknown in algae***

- *AVP1 overexpression increases carbohydrates under N deplete conditions*

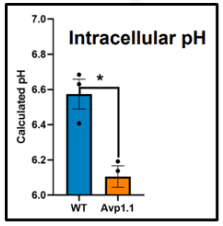


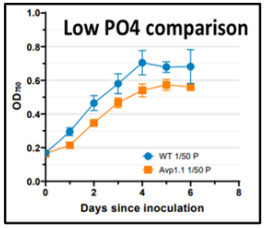




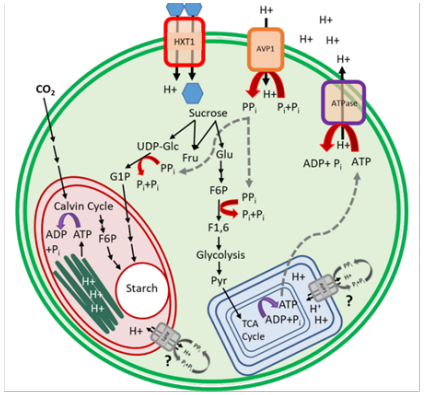
K Wright, R. Gonzalez, T. Dale

- *AVP1 overexpression decreases intracellular pH and diminishes growth on low PO_4 conditions*





- *AVP1 overexpression improves biochemical composition (increased C storage) of algal biomass, likely due to PP_i synthesis (provoking an intracellular P-limiting condition) .*



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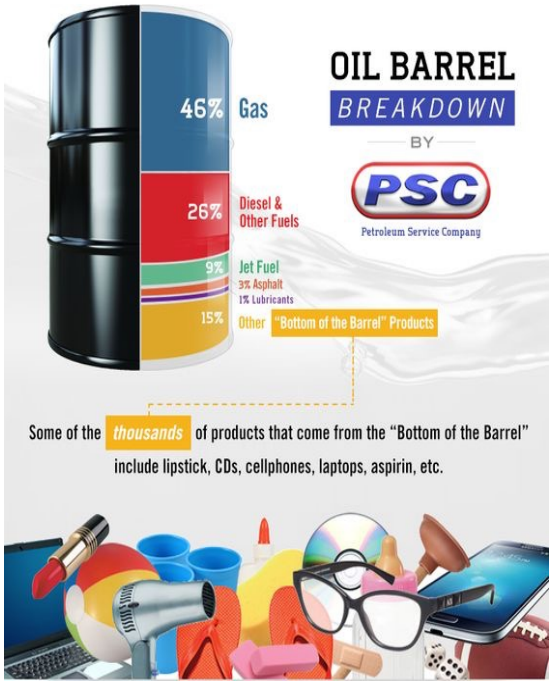
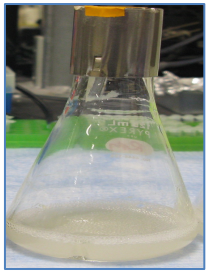
Biology leveraged as an alternative to petroleum

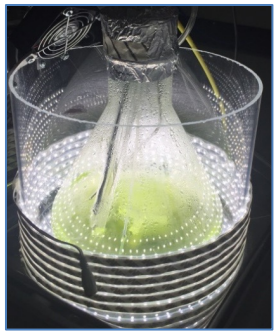


Source: Petroleum Service Company.
http://www.industrialoutpost.com/oil-barrel-42-gallon-breakdown/

PLANTS

Plants have been grown for ages, however, engineering can be challenging. Also, they can compete for land with food production.





ALGAE

Algae are fast growers and capable of fixing their own carbon, however, we still have yet to develop methods to efficiently utilize all biomass fractions.

BACTERIA

Bacteria are fast growers and easy to engineer, however, they require exogenous carbon supply (i.e. sugars, generally obtained from plants).

Los Alamos NATIONAL LABORATORY EST. 1943

GRACIAS

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